



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Mycobacterium bovis genomics reveals transmission of infection between cattle and deer in Ireland

Citation for published version:

Crispell, J, Cassidy, S, Kenny, K, McGrath, G, Warde, S, Cameron, H, Rossi, G, MacWhite, T, White, PCL, Lycett, S, Kao, RR, Moriarty, J & Gordon, SV 2020, 'Mycobacterium bovis genomics reveals transmission of infection between cattle and deer in Ireland', *Microbial Genomics*. <https://doi.org/10.1099/mgen.0.000388>

Digital Object Identifier (DOI):

[10.1099/mgen.0.000388](https://doi.org/10.1099/mgen.0.000388)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Microbial Genomics

Publisher Rights Statement:

This is an open-access article distributed under the terms of the Creative Commons Attribution License.

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Mycobacterium bovis genomics reveals transmission of infection between cattle and deer in Ireland

Joseph Crispell^{1,2,*}, Sophie Cassidy¹, Kevin Kenny³, Guy McGrath⁴, Susan Warde³, Henrietta Cameron³, Gianluigi Rossi^{5,6}, Teresa MacWhite⁷, Piran C. L. White⁸, Samantha Lycett⁶, Rowland R. Kao^{5,6}, John Moriarty³ and Stephen V. Gordon^{1,9}

Abstract

Control of bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, in the Republic of Ireland costs €84 million each year. Badgers are recognized as being a wildlife source for *M. bovis* infection of cattle. Deer are thought to act as spillover hosts for infection; however, population density is recognized as an important driver in shifting their epidemiological role, and deer populations across the country have been increasing in density and range. County Wicklow represents one specific area in the Republic of Ireland with a high density of deer that has had consistently high bTB prevalence for over a decade, despite control operations in both cattle and badgers. Our research used whole-genome sequencing of *M. bovis* sourced from infected cattle, deer and badgers in County Wicklow to evaluate whether the epidemiological role of deer could have shifted from spillover host to source. Our analyses reveal that cattle and deer share highly similar *M. bovis* strains, suggesting that transmission between these species is occurring in the area. In addition, the high level of diversity observed in the sampled deer population suggests deer may be acting as a source of infection for local cattle populations. These findings have important implications for the control and ultimate eradication of bTB in Ireland.

DATA SUMMARY

All whole-genome sequence data used for our analyses have been uploaded to the National Center for Biotechnology Information Sequence Read Archive (NCBI-SRA) under BioProject number PRJNA589836: www.ncbi.nlm.nih.gov/bioproject/PRJNA589836. Due to the sensitivity of the associated metadata, only the sampling date and species are provided with these sequences. All the code generated for this manuscript is freely available on GitHub: scripts to process the whole-genome sequencing data <https://github.com/JosephCrispell/GeneralTools/tree/master/Processing-Pipeline>; and scripts used to analyse the processed genomic data – <https://github.com/JosephCrispell/GeneralTools/tree/master/RepublicOfIreland/Wicklow>.

INTRODUCTION

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, affects cattle populations around the world [1–4]. In many countries with endemic bTB, wildlife play a role in the spread and persistence of *M. bovis* infection in cattle, hence, complicating bTB control [3, 5–8].

In the Republic of Ireland, control of bTB currently costs farmers, the exchequer and the European Union €84 million per year [9]. Populations of the European badger (*Meles meles*) can maintain *M. bovis* and act as a source of infection for cattle [10, 11]. As a result, badger populations across the country are managed as part of the national bTB control programme [12]. While deer are susceptible to infection, their role in *M. bovis* spread and persistence is uncertain due to

Received 11 December 2019; Accepted 19 May 2020; Published 18 June 2020

Author affiliations: ¹School of Veterinary Medicine, University College Dublin, Dublin, Ireland; ²Data Science Campus, Office for National Statistics, Newport, UK; ³Central Veterinary Research Laboratory, Backweston, County Kildare, Ireland; ⁴UCD Centre for Veterinary Epidemiology and Risk Analysis (CVERA), School of Veterinary Medicine, University College Dublin, Dublin, Ireland; ⁵Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK; ⁶Roslin Institute, University of Edinburgh, Edinburgh, UK; ⁷Department of Agriculture, Food and the Marine, Backweston, County Kildare, Ireland; ⁸Department of Environment and Geography, University of York, Wentworth Way, York YO10 5NG, UK; ⁹UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland.

*Correspondence: Joseph Crispell, crispelljoseph@gmail.com

Keywords: bovine tuberculosis; *Mycobacterium bovis*; deer; badger; Wicklow; phylogenetics.

Abbreviations: bTB, bovine tuberculosis; DAFM, Department of Agriculture, Food and the Marine; NCBI-SRA, National Center for Biotechnology Information Sequence Read Archive; SNV, single nucleotide variant.

Whole-genome sequence data has been uploaded to the NCBI-SRA under BioProject number PRJNA589836.

Data statement: All supporting data, code and protocols have been provided within the article or through supplementary data files. Two supplementary figures are available with the online version of this article.

000388 © 2020 The Authors



This is an open-access article distributed under the terms of the Creative Commons Attribution License.

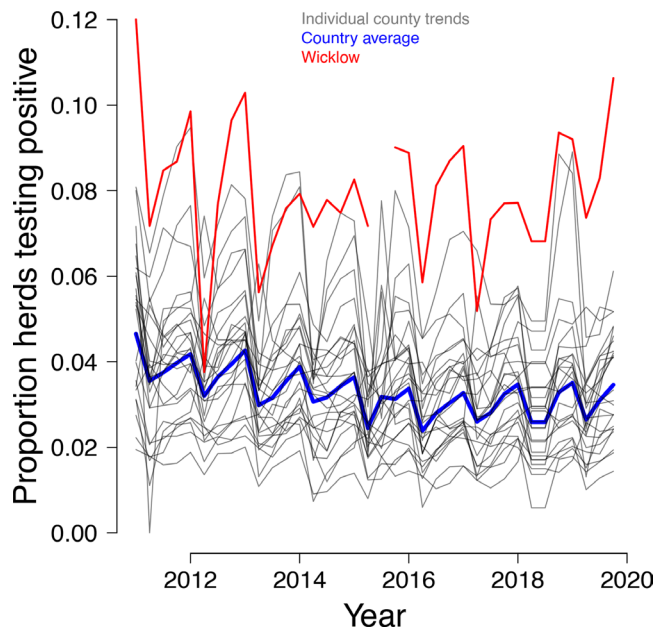


Fig. 1. Proportion of herds in each county of the Republic of Ireland testing positive for bovine tuberculosis from 2011 to 2020. Source: 'Bovine Tuberculosis by Regional Veterinary Offices, Year and Statistic (2010-498 2019)'.

insufficient data, and deer are not managed nationally under the bTB control programme [13–15].

The epidemiological role of deer in bTB, i.e. whether they are spillover hosts or a source of infection, is known to be linked to population density [7, 16–21]. Infection outcomes in deer range from a relatively common presentation of minimal pathology with infected deer living for many years, to a rarer chronic generalized infection involving multiple organ systems and a high fatality rate [7, 22–25]. Across Europe, deer species such as red deer and fallow deer are known to act as sources of infection for cattle in localized areas of high density, or as part of a multi-host wildlife reservoir [17, 18, 26, 27]. In Ireland, bTB outbreaks in Irish farmed deer have also been documented [28]. One bTB 'hot-spot' in Ireland is County Wicklow, where high densities of deer have been implicated in the local spread and persistence of *M. bovis* infection in cattle [29]. Furthermore, the range and density of wild-deer populations in Ireland is increasing [30, 31]. These increases highlight the need to quantify the role of deer in bTB epidemiology [32].

Whole-genome sequencing of *M. bovis* has been used to track transmission within and between cattle and wildlife populations [21, 33–37]. These studies have demonstrated that genomics adds unprecedented resolution, in comparison to previous molecular-typing technologies, in many cases distinguishing infection between individual animals. With Ireland seeking eradication of bTB by 2030 [9], the additional resolution of genomics could provide critical insights about

Impact Statement

In the Republic of Ireland, bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, threatens the sustainability of cattle production, with bTB control costing the government and industry €84million per year. Whilst badgers are recognized as being a source of infection for cattle, similar evidence on the role of deer in Ireland is lacking – despite the known susceptibility of deer to *M. bovis*. Whole-genome sequencing of *M. bovis* has previously been used to elucidate the role of different host species in multi-host pathogen transmission systems. Here, we use whole-genome sequencing of *M. bovis* sourced from infected cattle, badgers and deer to investigate the role of deer in the spread and persistence of *M. bovis* infection in a bTB hotspot in County Wicklow, Ireland. Our analyses suggest that *M. bovis* is transmitted between cattle and deer populations, and that deer may be acting as an important source of infection in the area. As such, *M. bovis* genome sequencing can shed new light on *M. bovis* transmission and provide quantitative data to support bTB policy formulation.

transmission within and between cattle and wildlife populations, and hence serve to support and refine bTB control policy.

A key question in resolving the current bTB hotspot in County Wicklow is to establish whether wild deer are involved in the spread and persistence of *M. bovis* in the local cattle population. Herein, we describe the application of whole-genome sequencing of *M. bovis* sampled from infected cattle, badgers and deer taken from a 100km² area in County Wicklow to directly address this question.

METHODS

Sample selection

In the last decade, County Wicklow has frequently had the highest herd-level prevalence of bTB in the Republic of Ireland, as shown by Fig. 1 [38, 39]. The Irish Department of Agriculture, Food and the Marine (DAFM) conducted a research study in County Wicklow in 2014 and 2015 that aimed to establish the prevalence of *M. bovis* infection in the local deer population (J Moriarty and others, unpublished data). During this study, culling operations were conducted in the deer and badger populations, while cattle herds in the area underwent statutory bTB testing. *M. bovis* culture was performed using MGITs (mycobacteria growth indicator tubes) from post-mortem samples of the culled badgers and deer, and test-positive cattle. Positive cultures were archived at the DAFM Central Veterinary Research Laboratory (Backweston, Ireland).

Frozen isolates from cattle, deer and badgers within the selected time frame were located in the DAFM Central

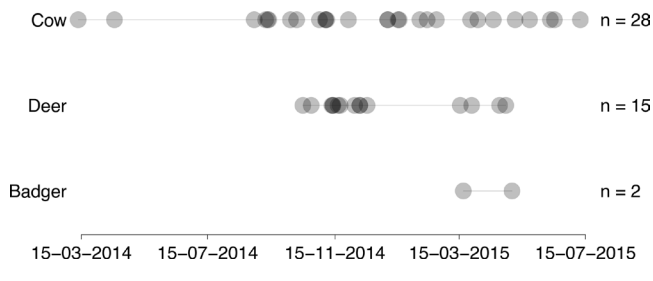


Fig. 2. Sampling dates for the *M. bovis* samples available from the Wicklow area. Shading is darker where circles overlap.

Veterinary Research Laboratory archives and re-cultured for sequencing. While the majority of deer and cattle isolates were resuscitated, only a minority of badger isolates could be recovered. The sequenced isolates represent all deer and badger isolates that could be recovered from the biobank, which were originally collected from 133 deer (23 infected on culture) and 68 badgers (17 infected on culture). The 28 cattle isolates that were sequenced were sampled from a total of 274 cattle isolates from this region, from which 174 isolates were available, with a single isolate from each herd selected for whole-genome sequencing; this isolate was from a home-bred animal or an animal that had been in the herd for several years. Hence, in total, 45 *M. bovis* isolates were successfully re-cultured from 28 cattle, 15 deer (14 sika and 1 fallow) and 2 badgers, sampled from 2014 to 2015 (Fig. 2). All the samples were sourced from animals present within an area of approximately 100 km², equating to approximately 5% of the total area (2027 km²) of County Wicklow (Fig. 3). Wildlife locations were provided as coordinates of the location where the animal was shot or trapped (DAFM). Cattle locations were derived from land-parcel data associated with each sampled herd available from the DAFM Land Parcel Identification System (LPIS). Cattle testing information was available through the DAFM Animal Health Computer System (AHCS).

Whole-genome sequencing data – generation and processing

DNA was extracted from the cultured *M. bovis* isolates using an AMPure XP magnetic bead based extraction protocol [40] and sequenced at the UCD Conway Institute Genomics Core (Dublin, Ireland) using an Illumina NextSeq system, producing 2×150bp paired-end reads. The raw sequencing data was assessed using FASTQC (v0.11.2; RRID:SCR_014583) [41]. The sequencing reads were trimmed, and adapters were removed where present using Cutadapt (v1.18; RRID:SCR_011841) [42]. Trimmed reads were aligned against the *M. bovis* reference genome (AF2122/97) [43] using the MEM tool from BWA (Burrows–Wheeler aligner) (v0.7.17; RRID:SCR_010910) [44]. Any annotated repeat regions, or those encoding proline-glutamic acid (PE) and proline-proline-glutamic acid (PPE) proteins, were excluded [45]. Excluding the single nucleotide variants (SNVs) within the PE and PPE

regions was found to have no influence on the phylogenetic relationship reported in the current research (Supplementary Figs 1 and 2, available with the online version of this article). For the aligned sequence data, SNVs were recorded if they had mapping quality ≥ 30 , high-quality base depth ≥ 4 on the forward and ≥ 4 on the reverse reads, read depth ≥ 30 reads and allele support ≥ 0.95 . If a site failed these criteria and the allele called was observed in each isolate's sequence data, it was accepted if it had a total high-quality base depth ≥ 4 and allele support ≥ 0.95 . Any SNVs within 10 bp of one another were removed to avoid regions of the genome that were prone to sequencing errors or under high selection. All the genomes were *in silico* spoligotyped using the *SpoTyping* tool (v2.0; RRID:SCR_018466) [46].

Phylogeny reconstruction

A maximum-likelihood phylogeny was reconstructed with RAxML (v8.2.11; RRID:SCR_006086) [47] using an alignment based on the concatenated SNVs from each sequenced isolate with a generalized time-reversible (GTR) substitution model [48]. The phylogeny was visualized in the statistical programming environment R (v3.6.1) [49] using the APE package (v5.0; RRID:SCR_017343) [50].

Clustering

The extent of species-level clustering in the genetic distances between the *M. bovis* genomes was investigated. Genetic distances were calculated by counting the number of differences between each pair of concatenated SNV sequences. These genetic distances were then divided into within- and between-species categories and compared.

RESULTS

Whole-genome sequencing

High-quality sequencing data was generated for all 45 *M. bovis* isolates [on average, each genome had 99% coverage (lower 2.5%, 0.82; upper 97.5%, 0.99) of its genome with a read depth ≥ 20 reads]. Spoligotypes could be reconstructed from the whole-genome sequence data and all isolates were type SB00054.

Phylogeny in space

All the *M. bovis* genomes sourced from infected cattle, badgers and deer in the Wicklow area were within 35 SNVs of one another (median distance=14 SNVs; lower 2.5%, 1; upper 97.5%, 30) (Fig. 4). There were multiple instances of *M. bovis* genomes sourced from cattle and wildlife being less than three SNVs apart, a distance that, in the human field, is indicative of recent transmission of *Mycobacterium tuberculosis* [51]. The deer-derived *M. bovis* genomes had the highest genomic diversity, with representatives found across the phylogeny as well as in a distinct single-species clade (labels 42–45 in Fig. 4).

The approximate sampling locations for the cattle, badgers and deer were all within 17 km of one another (median distance, 6.7 km; lower 2.5%, 1.1; and upper 97.5%, 12.9).

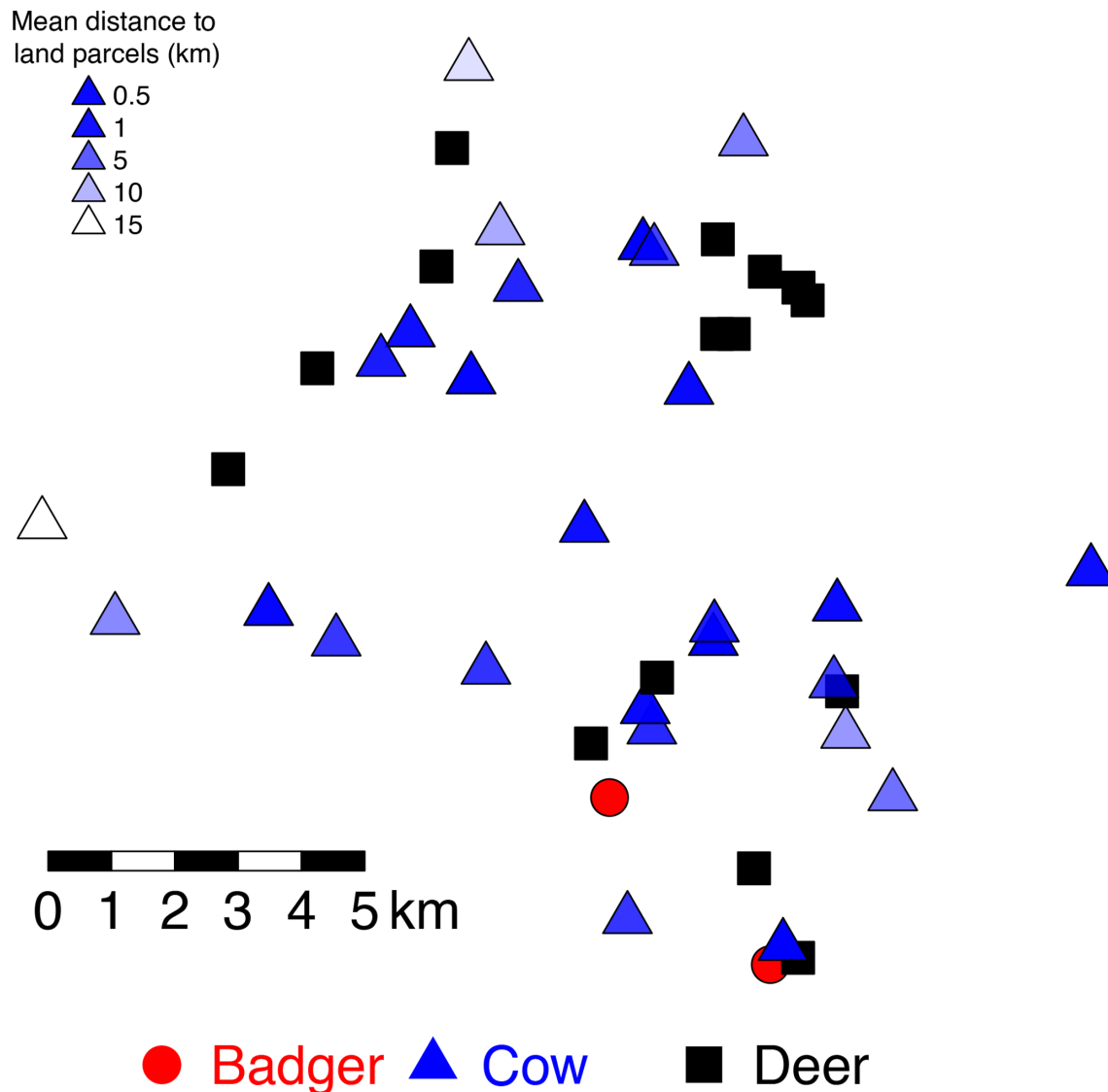


Fig. 3. Sampling locations for the *M. bovis* isolates from the Wicklow area. Each point represents the capture location for deer, the sett for badgers and the approximate herd locations for cattle [the latter to protect the identity of farm owners in compliance with GDPR (General Data Protection Regulation)]. The transparency of shading for cattle locations illustrates our certainty about where the sampled cow resided: the more transparent the triangle the more distant the herd's land parcels were from the approximate location.

The polygons in Fig. 4 highlight where animals that were infected with highly similar strains (≤ 3 SNVs) of *M. bovis* were found in close proximity (≤ 2.5 km) to one another. Only four small clusters were identified, suggesting that, in general, animals sharing similar *M. bovis* were not sampled close to one another. One of the clusters identified contained both cattle and deer (labels 2–5 and 7 in Fig. 4).

Patterns of clustering

There was no evidence of species-specific clustering in the genetic distance distribution, since there was considerable overlap between all the within- and between-species genetic

distance distributions (Fig. 5). The multiple instances of cattle- and wildlife-derived *M. bovis* genomes being highly similar (< 3 SNVs) are shown in the badger–cattle and cattle–deer subsets of the genetic distance distribution.

DISCUSSION

Our research used *M. bovis* whole-genome sequence data to address whether deer in County Wicklow have an important epidemiological role in bTB in cattle. Analysis of the *M. bovis* genomes sourced from cattle, badgers and deer found that all species shared highly similar strains. Our data are limited,

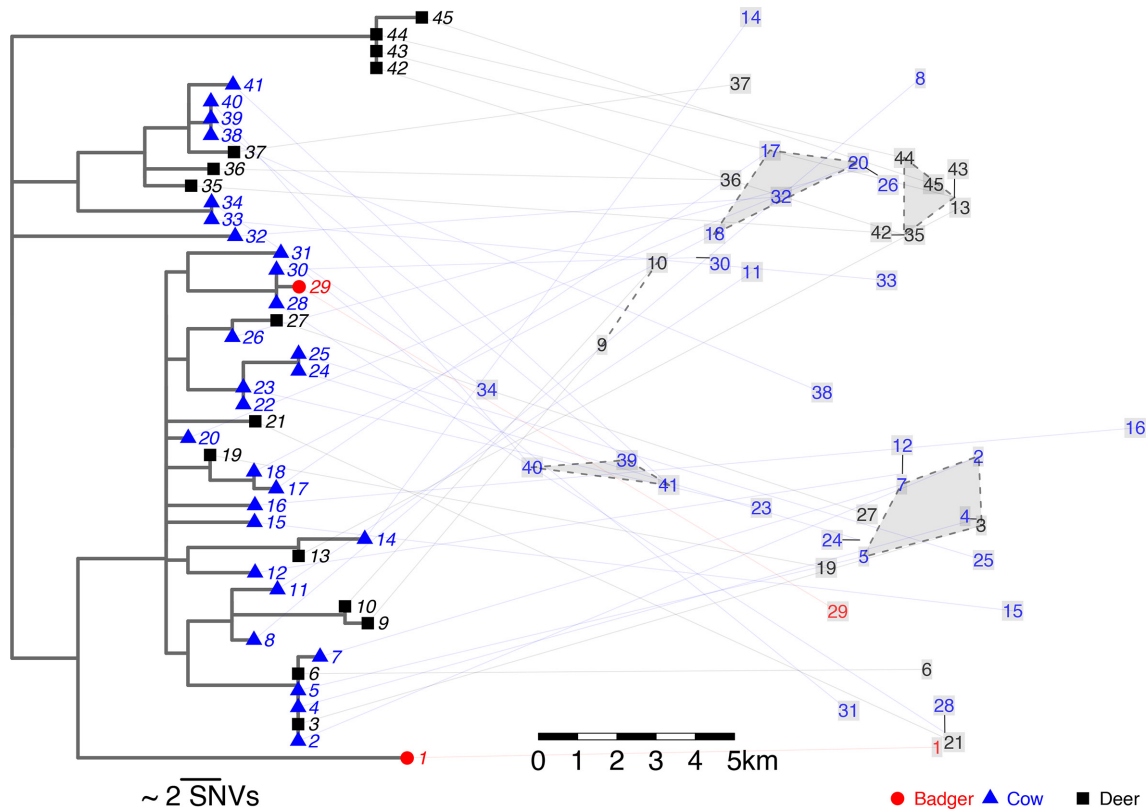


Fig. 4. A maximum-likelihood phylogeny built with RAxML (v8.2.11) [47] and rooted using AF2122/97 (*M. bovis* reference genome) [43]. Each of the tips is linked via a line to its sampling location. The sampling locations are plotted as the indices of the tip in the phylogeny. Some sampling locations were slightly repositioned to avoid overlapping labels using the basicPlotter R package (<https://github.com/JosephCrispell/basicPlotter>). Grey polygons highlight clusters of genomes with three or less differences and with approximate sampling locations within 2.5 km.

and the following interpretation is, therefore, framed within these constraints.

The high similarity of the *M. bovis* genomes sourced from cattle, badgers and deer suggests that in the sampled area all three host species are involved in the spread and persistence of *M. bovis* (Fig. 3). While badgers are a recognized source of *M. bovis* for cattle, and the presence of a badger-derived *M. bovis* genome only one SNV from two cattle-derived strains supports this (Fig. 5), the availability of only two *M. bovis* isolates from badgers limits our ability to further examine their role. In contrast, the larger number of samples from deer presents evidence suggesting recent transmission between cattle and deer, with 5 of the 15 *M. bovis* genomes sourced from deer being within three or less SNVs of those sourced from cattle (Figs 4 and 5). Importantly, such similarity could result from a common source, such as badgers. Defining the role of deer in the bTB system in Wicklow will require further research for which our study provides the baseline.

Despite having 28 genomes sourced from cattle, there was more diversity between the deer-derived *M. bovis* genomes (Fig. 5). Within-species diversity is commonly used to evaluate the epidemiological role of species, with high diversity

suggesting the species is acting as a source of infection [52]. While concluding that deer are acting as a source population in County Wicklow would rely upon representative sampling of each host species, which was not possible here, the diversity of *M. bovis* in deer suggests they could be playing an important role in the spread and persistence of infection in the area.

If deer are playing a role in the cattle and wildlife *M. bovis* transmission systems, local persistence of *M. bovis* in wildlife could be prolonged and infection spread further. Sika and fallow deer can live up to twice as long as badgers (12–16 years versus 5–8 years in badgers) and range over considerably larger distances (mean home-range size of 0.5–10 km² versus typically less than 500 m for badgers) [53–57]. These large ranging distances could explain the spatial clustering shown in Fig. 4, which suggests that the *M. bovis* strains present in the area are not spatially constrained. The aggregation of cattle, deer and badgers into herds and social groups means infection can persist locally. However, deer are recognized as important spatial vectors for *M. bovis* spread [7, 13, 20, 58–60] and their movements could be spreading *M. bovis* within the sampling area, hence, reducing patterns of spatial localization.

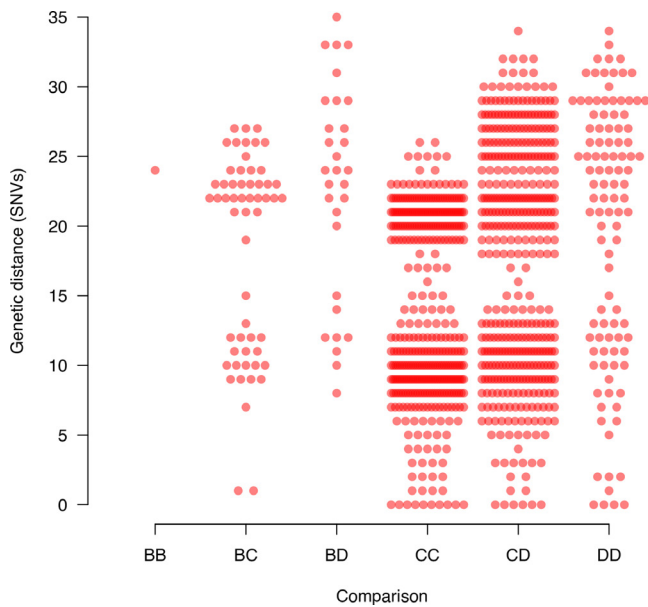


Fig. 5. Comparing the genetic distances within and between species. The genetic distance distribution for the *M. bovis* genomes was subdivided into distances associated with badger–badger (BB), badger–cattle (BC), badger–deer (BD), cattle–cattle (CC), cattle–deer (CD) and deer–deer (DD) comparisons. The raw data were overlaid using the spreadPoints() function in the basicPlotter R package (<https://github.com/JosephCrispell/basicPlotter>).

Combating pathogen transmission in a multi-host system requires knowledge of each host species' role [61]. Our research shows how *M. bovis* genome sequencing has the potential to provide new and detailed insights into local transmission dynamics in the bTB system. In Ireland, badgers are known to be a maintenance host of *M. bovis* infection. Our current research suggests that in County Wicklow deer could also be acting as a source of infection for cattle, potentially as part of a multi-host wildlife reservoir similar to those that exist with wild boar [26]. Therefore, our research highlights the need for surveillance to extend to deer populations in areas of high density across Ireland and provides a compelling case for the integration of genomics into routine bTB surveillance.

Funding information

This publication has emanated from research supported in part by a joint Biotechnology and Biological Sciences Research Council (BBSRC)–Science Foundation Ireland (SFI) research grant from SFI under grant number SFI/16/BBSRC/3390 (J.C., S.C., S.V.G.) and the BBSRC under grant reference BB/P010598/1 (G.R., P.C.L.W., S.L. and R.R.K.). S.L. is supported by a BBSRC institute strategic programme grant to the Roslin Institute, Control of Infectious Diseases – Pathogen Diversity, Host Specificity and Virulence (BBS/E/D/20002173), and the Scottish Government Rural and Environment Science and Analytical Services Division, as part of the Centre of Expertise on Animal Disease Outbreaks (EPIC). S.L. was also supported by a University of Edinburgh Chancellor's Fellowship. The funders had no role in study design, data collection and analysis, decision to publish nor preparation of the manuscript.

Acknowledgements

We wish to acknowledge Eamonn McDonald, Jack McGuirk and Sean Creane for their support in wildlife sampling; Alison Murphy and John Browne and the UCD Conway Genomics Core facility for help with DNA sequencing; and Eoin Ryan, Philip Breslin and Liam Barry for support and discussion. We would also like to thank Simon More for advice during manuscript preparation.

Author contributions

J.C., analysed the data and wrote the manuscript. S.C., performed the DNA extractions and whole-genome sequencing. T.M., was the veterinary inspector in Wicklow who identified and visited all the cattle farms and co-ordinated the collection of samples from test-positive cattle and culled badgers in the area. G.M., provided advice on analyses, access to cattle and wildlife population data. G.R., P.C.L.W., S.L. and R.R.K., provided advice on the analyses. K.K., S.W., H.C. and J.M., handled the collection and processing of samples and culturing of *M. bovis*. S.V.G. and R.R.K., sourced the funding. S.V.G., oversaw the project and helped write the manuscript. All authors provided comments on the draft of the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Data Bibliography

1. Crispell J, Cassidy S, Kenny K, McGrath G, Warde S, Cameron H, Rossi G, MacWhite T, White PCL, Lycett S, Kao RR, Moriarty J, Gordon SV. Whole-genome sequence data, NCBI-SRA BioProject number PRJNA589836 (www.ncbi.nlm.nih.gov/sra/PRJNA589836) (2019).
2. Crispell J. GitHub, scripts to process the whole-genome sequencing data – <https://github.com/JosephCrispell/GeneralTools/tree/master/ProcessingPipeline> (2017).
3. Crispell J. GitHub, scripts to analyse the processed genomic data – <https://github.com/JosephCrispell/GeneralTools/tree/master/RepublucOfIreland/Wicklow> (2020).

References

1. Ayele WY, Neill SD, Zinsstag J, Weiss MG, Pavlik I *et al.* Bovine tuberculosis: an old disease but a new threat to Africa. *Int J Tuberc Lung Dis* 2004;8:924–937.
2. Godfray C, Donnelly C, Hewinson G, Winter M, Wood J. *Bovine TB Strategy Review* (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/756942/tb-review-final-report-corrected.pdf). London: Department for Environment, Food and Rural Affairs; 2018.
3. Reviriego Gordejo FJ, Vermeersch JP. Towards eradication of bovine tuberculosis in the European Union. *Vet Microbiol* 2006;112:101–109.
4. de Kantor IN, Ritacco V. An update on bovine tuberculosis programmes in Latin American and Caribbean countries. *Vet Microbiol* 2006;112:111–118.
5. Godfray HCJ, Donnelly CA, Kao RR, MacDonald DW, McDonald RA *et al.* A restatement of the natural science evidence base relevant to the control of bovine tuberculosis in Great Britain. *Proc Biol Sci* 2013;280:20131634.
6. Miller RS, Sweeney SJ. *Mycobacterium bovis* (bovine tuberculosis) infection in North American wildlife: current status and opportunities for mitigation of risks of further infection in wildlife populations. *Epidemiol Infect* 2013;141:1357–1370.
7. Nugent G, Gortazar C, Knowles G. The epidemiology of *Mycobacterium bovis* in wild deer and feral pigs and their roles in the establishment and spread of bovine tuberculosis in New Zealand wildlife. *N Z Vet J* 2015;63 (Suppl. 1):54–67.
8. Payeur JB, Church S, Mosher L, Robinson-Dunn B, Schmitt S *et al.* Bovine tuberculosis in Michigan wildlife. *Ann N Y Acad Sci* 2002;969:259–261.
9. DAFM. *Bovine TB Stakeholder Forum* (<https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/diseasecontrols/tuberculosisandbrucellosis/tbforum/TBForumConsultationPaper%20s140818.pdf>). Dublin: Department of Agriculture, Food and the Marine, Ireland; 2018.

10. Byrne AW, Kenny K, Fogarty U, O'Keeffe JJ, More SJ *et al.* Spatial and temporal analyses of metrics of tuberculosis infection in badgers (*Meles meles*) from the Republic of Ireland: trends in apparent prevalence. *Prev Vet Med* 2015;122:345–354.
11. More SJ, Good M. Understanding and managing bTB risk: perspectives from Ireland. *Vet Microbiol* 2015;176:209–218.
12. DAFM. *Ireland's Bovine TB Eradication Programme: 2018 Overview* (<https://www.agriculture.gov.ie/animalhealthwelfare/diseasecontrol/bovinetb/diseaseeradicationtb/irelandsbovinetberadicationprogramme/>). Dublin: Department of Agriculture, Food and the Marine, Ireland; 2018.
13. Delahay RJ, De Leeuw ANS, Barlow AM, Clifton-hadley RS, Cheeseman CL. The status of *Mycobacterium bovis* infection in UK wild mammals: a review. *Vet J* 2002;164:90–105.
14. Gormley E, Collins JD. The development of wildlife control strategies for eradication of tuberculosis in cattle in Ireland. *Tuber Lung Dis* 2000;80:229–236.
15. More SJ, Good M. The tuberculosis eradication programme in Ireland: a review of scientific and policy advances since 1988. *Vet Microbiol* 2006;112:239–251.
16. Busch F, Bannerman F, Liggett S, Griffin F, Clarke J *et al.* Control of bovine tuberculosis in a farmed red deer herd in England. *Vet Rec* 2017;180:68.
17. Delahay RJ, Smith GC, Barlow AM, Walker N, Harris A *et al.* Bovine tuberculosis infection in wild mammals in the south-west region of England: a survey of prevalence and a semi-quantitative assessment of the relative risks to cattle. *Vet J* 2007;173:287–301.
18. Gortázar C, Delahay RJ, McDonald RA, Boadella M, Wilson GJ *et al.* The status of tuberculosis in European wild mammals. *Mamm Rev* 2012;42:193–206.
19. Hermoso de Mendoza J, Parra A, Tato A, Alonso JM, Rey JM *et al.* Bovine tuberculosis in wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) and cattle (*Bos taurus*) in a Mediterranean ecosystem (1992–2004). *Prev Vet Med* 2006;74:239–247.
20. O'Brien DJ, Schmitt SM, Fitzgerald SD, Berry DE. Management of bovine tuberculosis in Michigan wildlife: current status and near term prospects. *Vet Microbiol* 2011;151:179–187.
21. Sunstrum J, Shoyinka A, Power LE, Maxwell D, Stobierski MG *et al.* Notes from the field: zoonotic *Mycobacterium bovis* disease in deer hunters – Michigan, 2002–2017. *MMWR Morb Mortal Wkly Rep* 2019;68:807–808.
22. Fitzgerald SD, Kaneene JB. Wildlife reservoirs of bovine tuberculosis worldwide: hosts, pathology, surveillance, and control. *Vet Pathol* 2013;50:488–499.
23. Johnson LK, Liebana E, Nunez A, Spencer Y, Clifton-Hadley R *et al.* Histological observations of bovine tuberculosis in lung and lymph node tissues from British deer. *Vet J* 2008;175:409–412.
24. Palmer MV, O'Brien DJ, Griffin JF, Nugent G, de Lisle GW, *et al.* Tuberculosis in wild and captive deer. In: Mukundan H, Chambers MA, Waters WR, Larsen MH (editors). *Tuberculosis, Leprosy and Mycobacterial Diseases of Man and Animals: the Many Hosts of Mycobacteria*. Wallingford: CAB International; 2015. pp. 334–364.
25. Rhyan JC, Saari DA. A comparative study of the histopathologic features of bovine tuberculosis in cattle, fallow deer (*Dama dama*), sika deer (*Cervus nippon*), and red deer and elk (*Cervus elaphus*). *Vet Pathol* 1995;32:215–220.
26. Hardstaff JL, Marion G, Hutchings MR, White PCL *et al.* Evaluating the tuberculosis hazard posed to cattle from wildlife across Europe. *Res Vet Sci* 2014;97:S86–S93.
27. Ward AI, Smith GC, Etherington TR, Delahay RJ. Estimating the risk of cattle exposure to tuberculosis posed by wild deer relative to badgers in England and Wales. *J Wildl Dis* 2009;45:1104–1120.
28. Partridge T, Toolan D, Egan J, More S. Control of *Mycobacterium bovis* infection in two sika deer herds in Ireland. *Ir Vet J* 2008;61:27–32.
29. More SJ. Can bovine TB be eradicated from the Republic of Ireland? Could this be achieved by 2030? *Ir Vet J* 2019;72:3.
30. Carden RF, Carlin CM, Marnell F, McElholm D, Hetherington J *et al.* Distribution and range expansion of deer in Ireland. *Mamm Rev* 2011;41:313–325.
31. Liu Y, McCullagh A, Nieuwenhuis M. What factors affect national-scale deer population dynamics in the Republic of Ireland? *Scand J For Res* 2018;33:535–549.
32. DAFM. *Bovine TB Stakeholder Forum Interim Report – Disease Policy and Working in Partnership* (https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/diseasecontrols/tuberculosis/tban_dbrucellosis/tbforum/InterimReportOfTheTBForum230719.pdf). Dublin: Department of Agriculture, Food, and the Marine, Ireland; 2019.
33. Crispell J, Zadoks RN, Harris SR, Paterson B, Collins DM *et al.* Using whole genome sequencing to investigate transmission in a multi-host system: bovine tuberculosis in New Zealand. *BMC Genomics* 2017;18:180.
34. Glaser L, Carstensen M, Shaw S, Robbe-Austerman S, Wunschmann A *et al.* Descriptive epidemiology and whole genome sequencing analysis for an outbreak of bovine tuberculosis in beef cattle and white-tailed deer in northwestern Minnesota. *PLoS One* 2016;11:e0145735.
35. Kohl TA, Utpatel C, Niemann S, Moser I. *Mycobacterium bovis* persistence in two different captive wild animal populations in Germany: a longitudinal molecular epidemiological study revealing pathogen transmission by whole-genome sequencing. *J Clin Microbiol* 2018;56:e00302-18.
36. Perea Razo CA, Rodríguez Hernández E, Ponce SIR, Milián Suazo F, Robbe-Austerman S *et al.* Molecular epidemiology of cattle tuberculosis in Mexico through whole-genome sequencing and spoligotyping. *PLoS One* 2018;13:e0201981.
37. Salvador LCM, O'Brien DJ, Cosgrove MK, Stuber TP, Schooley AM *et al.* Disease management at the wildlife-livestock interface: using whole-genome sequencing to study the role of elk in *Mycobacterium bovis* transmission in Michigan, USA. *Mol Ecol* 2019;28:2192–2205.
38. More S. *The Bovine Tuberculosis Eradication Programme in Ireland* (https://data.oireachtas.ie/ie/oireachtas/committee/dail/32/joint_committee_on_agriculture_food_and_the_marine/submissions/2019/2019-02-26_opening-statement-professor-simon-more-director-of-the-ucd-centre-for-veterinary-epidemiology-and-risk-analysis-ucd_en.pdf). Dublin: Department of Agriculture, Food and the Marine, Ireland; 2019.
39. DAFM. 2019. Bovine tuberculosis by regional veterinary offices, quarter and statistic. https://statbank.cso.ie/px/pxeirestat/Database/eirestat/Animal%20Disease%20Statistics/Animal%20Disease%20Statistics_statbank.asp?sp=Animal%20Disease%20Statistics&Planguage=0&ProductID=DB_DA [accessed October 2019].
40. Votintseva AA, Pankhurst LJ, Anson LW, Morgan MR, Gascoyne-Binzi D *et al.* Mycobacterial DNA extraction for whole-genome sequencing from early positive liquid (MGIT) cultures. *J Clin Microbiol* 2015;53:1137–1143.
41. Andrews S. FastQC: a quality control tool for high throughput sequence data; 2010. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>
42. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* 2011;17:10–12.
43. Malone KM, Farrell D, Stuber TP, Schubert OT, Aebbersold R *et al.* Updated reference genome sequence and annotation of *Mycobacterium bovis* AF212/97. *Genome Announc* 2017;5:e00157-17.
44. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25:1754–1760.
45. Sampson SL. Mycobacterial PE/PPE proteins at the host-pathogen interface. *Clin Dev Immunol* 2011;2011:497203.
46. Xia E, Teo Y-Y, Ong RT-H. *SpoTyping*: fast and accurate *in silico* Mycobacterium spoligotyping from sequence reads. *Genome Med* 2016;8:19.

47. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
48. Tavaré S. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 1986;17:57–86.
49. R Core Team. R: a Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing; 2019. <https://www.R-project.org/>
50. Paradis E, Claude J, Strimmer K. Ape: analyses of phylogenetics and evolution in R language. *Bioinformatics* 2004;20:289–290.
51. Meehan CJ, Moris P, Kohl TA, Pečerska J, Akter S *et al.* The relationship between transmission time and clustering methods in *Mycobacterium tuberculosis* epidemiology. *EBioMedicine* 2018;37:410–416.
52. Croucher NJ, Didelot X. The application of genomics to tracing bacterial pathogen transmission. *Curr Opin Microbiol* 2015;23:62–67.
53. Byrne AW, Sleeman DP, O’Keeffe J, Davenport J. The ecology of the European badger (*Meles meles*) in Ireland: a review. *Biology and Environment: Proceedings of the Royal Irish Academy* 2012;112B:105–132.
54. Harris S, Yalden DW. *Mammals of the British Isles: Handbook*. London: The Mammal Society; 2008.
55. Roper TJ, Ostler JR, Conradt L. The process of dispersal in badgers *Meles meles*. *Mamm Rev* 2003;33:314–318.
56. Byrne AW, Quinn JL, O’Keeffe JJ, Green S, Sleeman DP *et al.* Large-scale movements in European badgers: has the tail of the movement kernel been underestimated? *J Anim Ecol* 2014;83:991–1001.
57. DAFM. *Deer Management in Ireland: a Framework for Action* (<https://www.agriculture.gov.ie/media/migration/forestry/deermanagement/DeerManagementIrelandAframeworkforAction040315.pdf>). Dublin: Department of Agriculture, Food and the Marine, Ireland; 2015.
58. Barron MC, Tompkins DM, Ramsey DSL, Bosson MAJ. The role of multiple wildlife hosts in the persistence and spread of bovine tuberculosis in New Zealand. *N Z Vet J* 2015;63 (Suppl. 1):68–76.
59. Nugent G. Maintenance, spillover and spillback transmission of bovine tuberculosis in multi-host wildlife complexes: a new Zealand case study. *Vet Microbiol* 2011;151:34–42.
60. Yockney IJ, Nugent G, Latham MC, Perry M, Cross ML *et al.* Comparison of ranging behaviour in a multi-species complex of free-ranging hosts of bovine tuberculosis in relation to their use as disease sentinels. *Epidemiol Infect* 2013;141:1407–1416.
61. Haydon DT, Cleaveland S, Taylor LH, Laurenson MK. Identifying reservoirs of infection: a conceptual and practical challenge. *Emerg Infect Dis* 2002;8:1468–1473.

Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.